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Original Articles

Mitotic regulator Nlp interacts with XPA/ERCC1 complexes and regulates nucleotide excision repair (NER) in response to UV radiation

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ABSTRACT

Cellular response to DNA damage, including ionizing radiation (IR) and UV radiation, is critical for the maintenance of genomic fidelity. Defects of DNA repair often result in genomic instability and malignant cell transformation. Centrosomal protein Nlp (ninein-like protein) has been characterized as an important cell cycle regulator that is required for proper mitotic progression. In this study, we demonstrate that Nlp is able to improve nucleotide excision repair (NER) activity and protects cells against UV radiation. Upon exposure of cells to UVC, Nlp is translocated into the nucleus. The C-terminus (1030–1382) of Nlp is necessary and sufficient for its nuclear import. Upon UVC radiation, Nlp interacts with XPA and ERCC1, and enhances their association. Interestingly, down-regulated expression of Nlp is found to be associated with human skin cancers, indicating that dysregulated Nlp might be related to the development of human skin cancers. Taken together, this study identifies mitotic protein Nlp as a new and important member of NER pathway and thus provides novel insights into understanding of regulatory machinery involved in NER.

Introduction

Ultraviolet (UV) irradiation has been linked to skin cancers, including non-melanoma and melanoma skin cancers [1–3]. The major DNA lesions induced by UVB or by high-energy artificial UVC irradiation are *cis-syn* cyclobutane pyrimidine dimers (CPDs) and pyrimidine (6-4) pyrimidone photoproducts [(6-4) photoproducts; (6-4) PPs]. Usually, CPDs are formed quite significantly much more than (6-4) PPs [4,5]. CPDs formation and repair are important in connection with carcinogenesis in human skin [6].

Nucleotide excision repair (NER) is the main biological event responsible for the removal of bulky, helix-distorting DNA lesions, induced by UV irradiation, environmental mutagens, and certain chemo-therapeutic agents [7,8]. The NER pathway consists of two sub-pathways of NER, global genome NER (GG-NER) and transcription-coupled NER (TC-NER), differing in damage recognition [8,9]. Both sub-pathways require the core NER factors, of which XPA is the central component as it interacts with TFIIH, RPA, XPC-RAD23B, DDB2, ERCC1-XPF, and PCNA proteins [7,10–16]. ERCC1-XPF is recruited to NER complexes by interaction with XPA to trigger the dual incision reaction [10,17].

Nlp (ninein-like protein) has been characterized as a centrosomal protein that is essential for proper mitotic events. Nlp is tethered to the centrosome by BRCA1 and is involved in microtubule organization in interphase cells by recruiting γ-TuRCs and displaced from maturing centrosome at G2/M transition to ensure mitotic spindle formation [18–22]. Its subcellular localization and protein stability are regulated by several crucial mitotic kinases, such as Plk1, Nek2, Cdc2 and Aurora B [18,20,23–26]. Deregulation of Nlp in cells results in aberrant spindle, chromosomal missegregation and multinucleus, abnormal cytokinesis and induces chromosomal instability and renders cell tumorigenesis [18,20,23,24,26].

Our previous studies have identified that Nlp interacts with BRCA1 through its C terminus, which is required for normal microtubule nucleation at interphase [22]. Since BRCA1 also functions in DNA damage response [27], we propose that Nlp might implicate in the process of DNA repair. In this work, we show that Nlp plays a role in NER after UV-induced DNA damage. More interestingly, downregulated expression of Nlp is observed in human skin carcinomas.

Materials and methods

H1299, A549 and HeLa cell lines were purchased from ATCC (American Type Culture Collection). They were cultured under desired medium. For UV treatment, exponentially growing cells or the cells after transfection were rinsed with phosphate-buffered saline (PBS) and irradiated with UV radiation at different doses. After UV irradiation, fresh medium was added and the cells were cultured until harvest. Detailed information is shown in the Supplementary materials.

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Results

Nlp enhances DNA repair following UV-induced DNA damage

In order to determine whether Nlp is involved in DNA damage response, we treated H1299 cells (lung cancer cell line) with IR or UV with appropiate doses. There was little change of subcellular localization of Nlp in the case of IR which mainly caused double strand breaks (DSBs) (data not shown). However, after UV radiation, the localization of Nlp changed obviously (Fig. 1A). Then we asked whether Nlp contributes to photoproducts excision. Host cell reactivation (HCR) assay and the removal of CPDs assay by ELISA were employed. Upon knockdown of Nlp expression in H1299 cell line (Fig. 1B), the HCR activity and the rate of CPDs removal were significantly attenuated compared with the control groups (Fig. 1C and D). Similarly, both the HCR activity and the removal of CPDs were more powerful in HeLa cells (cervical cancer cell line) reconstituted with pEGFP-C3-Nlp and KYSE30 cells (esophageal squamous cell carcinoma cell line) transfected with pcDNA3.1(+)-Nlp than their counterparts (Fig. 1E–G and Supplementary Fig. S4A–C). Consistently, when the removal of CPDs was measured in MEFs derived from Nlp transgenic [28] and wild type mice, similiar results were obtained (Fig. 1H). Collectively, the findings indicate that Nlp is involved in the DNA repair process and probably regulates nucleotide excision repair (NER) pathway following UV-radiation.

Nlp protects cells from UV radiation-induced cell death

Given that Nlp promotes NER activity, we next explored its physiological functions. H1299 cells transfected with Nlp siRNAs, HeLa and KYSE30 cells overexpressing Nlp were treated by UVC radiation with the indicated doses (0 J/m^2, 5 J/m^2, 10 J/m^2, 20 J/m^2, 40 J/m^2). Cell survival was then measured at 24 hours (Fig. 2A and B and Supplementary Fig. S4D) or 42 hours (Supplementary Fig. S1A and B) post-UV. The results showed that knockdown of Nlp lowered the cell survival, while over-expression of Nlp significantly enhanced it, suggesting that Nlp could render cells more resistant to UVC-induced cell death.

We next examined the colony formation after UVC-irradiation with doses of 0 J/m^2, 2 J/m^2, 4 J/m^2, 6 J/m^2, 8 J/m^2, and found that upon UVC-irradiation, the cells with depleted expression of Nlp exhibited weaker ability of colony formation, consistent with the

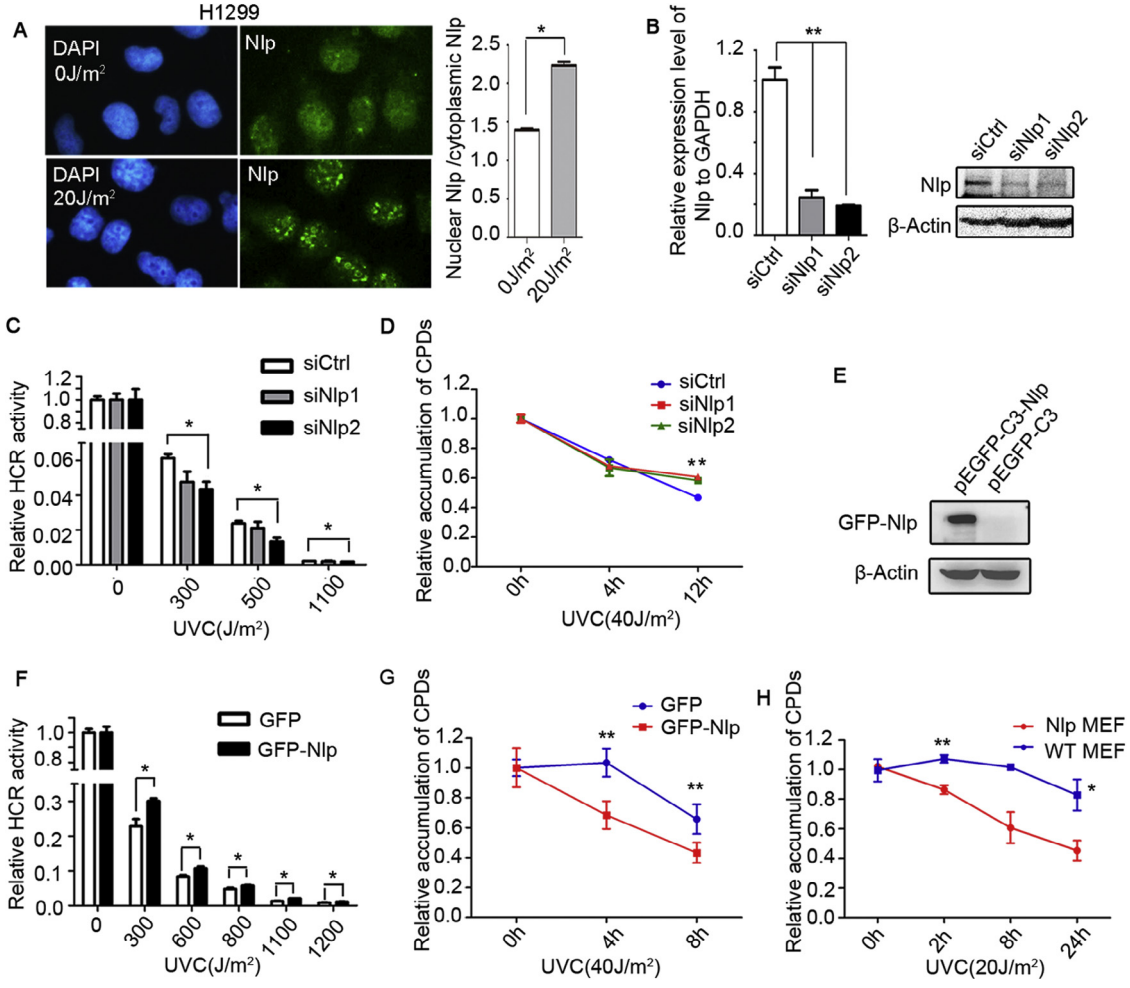

Fig. 1. Nlp positively regulates UV-induced DNA damage repair. (A) Upon UV treatment, Nlp subcellular localization was changed obviously. H1299 cells were exposed to UVC 20 J/m^2. After 4 hours, immunofluorescence assay was conducted. Quantification image (right) was obtained through analyzing anti-Nlp staining intensity of 500 cells. (B) Depleted Nlp levels after siRNA knockdown in H1299 were measured by real-time PCR and western blotting. (C and D) Knockdown of Nlp in H1299 reduced HCR activity (C) and removal efficiency of CPDs induced by UVC 40 J/m^2 (D). (E) Expression levels of GFP-Nlp in HeLa stable cell line were detected by western blotting. (F and G) Overexpression of Nlp in HeLa cells improved HCR activity (F) and removal efficiency of CPDs induced by UVC 40 J/m^2 (G). (H) In MEFs (derived from Nlp transgenic or wild type mice) examination, after UV 20 J/m^2 treatment, the removal efficiency of CPDs was higher in Nlp MEFs than WT MEFs. Removal efficiency of CPDs was measured by ELISA assay. All experiments were performed at least three times and data were statistically analyzed by a two-sided t-test. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs control. Nlp MEF, Nlp transgenic MEFs; WT MEF, wild type MEFs. Error bars indicate s.e.m.